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A MINI-REVIEW ON BIOCHEMICAL ASPECTS OF BILIRUBIN

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ABSTRACT

Bilirubin is the orange-yellow pigment derived from senescent red blood cells. It is transformed in the liver and is excreted in bile and urine. Bilirubin metabolism is very important for disposing of waste products generated by old red blood cells. First, the hemoglobin moves out of the heme molecule, which passes through a series of processes of porphyrin catabolism, depending on the part of the body where the breakdown occurs. The production of biliverdin from heme is the first major step in the catabolic pathway, after which the enzyme biliverdin reductase performs the second step, producing bilirubin from biliverdin. This review focuses on the basic chemical, biochemical, and disease aspects of bilirubin.

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1. INTRODUCTION

Virchow found bilirubin in blood extravasates in 1849 and named the yellow pigment "hematoidin." Stadeler first used the word "bilirubin" in 1864, and Tarchanoff showed that bile pigments directly interact with haemoglobin in 1874 [1]. Bilirubin IXa was created in 1942, and Fisher and Plieninger proposed its structure. For more than 30 years, the bilirubin molecule's linear tetrapyrrolic structure was acknowledged. However, the bilirubin molecule's insolubility in water and its solubility in several nonpolar solvents are crucial chemical characteristics [2]. This linear tetrapyrrole structure does not anticipate the solubility of bilirubin in nonpolar lipid solvents since the two propionic acid side chains would be expected to make the bilirubin molecule very polar and hence water-soluble. By using x-ray crystallography, the general chemical structure of bilirubin was determined [3]. This research suggests that bilirubin adopts a ridge-tiled structure supported by six intramolecular hydrogen bonds [4].

Additionally, two significant structural characteristics have been noted: 1) A double bond between carbons 4 and 5 and between 15 and 16 has a so-called Z-Z (Trans) conformation. 2) An involuted hydrogen-bonded structure in which the nitrogen atoms of the pyrrole rings are hydrogen-bonded to the propionic acid-carboxylic acid groups [5]. These connections maintain bilirubin's Z-Z structure and stop it from interacting with polar groups in watery conditions. When exposed to light, the Z-Z shape changes into the E-E (cis) conformation and other configurations, such as 4E-l 5Z and 4Z-l 5E. Since less internal hydrogen bonding occurs in the E-E conformation and other E-containing isomers than in the Z-Z conformation, they are more water soluble than the Z-Z conformation [6]. Bilirubin exposed to light is. Therefore more water soluble and easily eliminated in the bile. This justifies exposing neonates jaundiced to 450 nm light [7]. As previously indicated, the crystalline form of bilirubin has the shape of a ridge tile rather than a linear tetrapyrrole, with the ridge running along the line C8-C10-C12. With a 98° angle between the two rings, rings A and B are positioned

in one plane and rings C and D in another in this arrangement [8]. Although the optimal bilirubin conformation in an aqueous solution at pH 7.4 is unknown, the presence of a hydrogen-bound structure in an aqueous solution might help to explain some of the unusual chemical characteristics of bilirubin IXa [8].

For instance, for unconjugated bilirubin to react with diazo reagent, hydrogen bond-breaking substances such as caffeine, methanol, ethanol, urea, or surface active agents must be added. These substances most likely work by rupturing the bilirubin molecule's internal hydrogen bonds, allowing it to interact with diazotized sulfuric acid or other diazo compounds [9]. Contrarily, the monoglucuronide and diglucuronide forms of bilirubin IXa are soluble in water and easily react with diazo reagents. Conjugated bilirubin cannot generate internal hydrogen bonds because of the large glucuronic acid moiety. Unconjugated bilirubin is not easily eliminated in the bile or urine. However, bilirubin glucuronides are because they are water-soluble. Nearly all (99%) of the bilirubin derived from natural sources is the isomer IXa [10]. Less than 0.5 percent of bilirubin isolated from bile comprises bilirubins IX and IX8, which result from the cleavage of the 7- and 8-methene bridges. Illa and Xllla isomers of bilirubin are in varying amounts in reference materials for bilirubin that is available from commercial sources and the National Institute of Standards and Technology. Cleavage of bilirubin IXa at the central methylene bridge results in the formation of the two isomers; subsequent recombination of the two distinct dipyrrole units results in a mixture of the three isomers [10]. When bilirubin is not coupled to albumin, this isomerization of bilirubin does not occur in an aqueous solution at an acidic or neutral pH.

2. BIOCHEMISTRY OF BILIRUBIN

A microsomal heme oxygenase breaks down protoporphyrin IX to generate bilirubin IXa 1. The green pigment biliverdin, produced by the ring opening at the a- methene bridge, is reduced to bilirubin by the cytosolic enzyme biliverdin reductase from the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). This route produces one mole of carbon monoxide, bilirubin, and ferric iron for every mole of heme that is catabolized. A man's total daily bilirubin production is between 250 and 300 mg. The heme moiety of haemoglobin released by senescent erythrocytes that are degraded in the reticuloendothelial cells of the liver, spleen, and bone marrow accounts for around 85% of the total amount of bilirubin generated [11]. The remaining 15% is created by the degradation of other heme-containing proteins, such as myoglobin, cytochromes, and peroxidases, as well as the destruction of RBC precursors in the bone marrow (referred to as inefficient erythropoiesis).

3. TRANSPORT OF BILIRUBIN

Bilirubin is delivered to the liver by albumin-bound bilirubin in the blood. Bilirubin is bound loosely by albumin. Therefore, bilirubin may easily separate from albumin when it is in excess. Aspirin, penicillin, etc., can bind to albumin's bilirubin binding sites. Therefore, such medicines may deprive albumin of bilirubin [12]. Therefore, caution should be exercised while giving these medications to newborns to prevent kernicterus.

4. UPTAKE OF BILIRUBIN

The bilirubin is absorbed when the albumin-bilirubin complex reaches the sinusoidal surface of the liver. The carrier mediates the active mechanism of uptake. Bilirubin is reversibly linked to soluble proteins called ligandins or protein Y once it has entered the liver cells. Ligandins, which make up about 5% of the total protein in the cytosol of the human liver, are cytosolic proteins that belong to the glutathione-S transferase gene family. Other substances that ligandin binds include steroids, bromsulphthalein, indocyanine green, and certain carcinogens [13]. Ligandin plays a significant part in the processing of these chemicals; delaying the reflux of these molecules back into the plasma may boost net absorption efficiency.

5. CONJUGATION OF BILIRUBIN BY LIVER CELLS

Bilirubin is nonpolar; if it weren't changed into a more water-soluble form, it would stay in cells. The conjugation of bilirubin with glucuronic acid results in forming a more polar molecule. Bilirubin is quickly conjugated with glucuronic acid inside the hepatocytes to create bilirubin monoglucuronide and diglucuronide, which are then eliminated in the bile [14]. The creation of bilirubin monoglucuronide is catalyzed by the microsomal enzyme bilirubin uridine diphosphate (UDP) glucuronyltransferase (EC 2.4.1.17). It is unclear if the same enzyme, or another enzyme present in or close to the canaliculus, catalyzes the conversion of monoglucuronide to diglucuronide.

6. SECRETION OF BILIRUBIN

It is believed that the excretion of conjugated bilirubin into bile, which occurs in the face of a clear concentration gradient, is an energy-dependent active transport mechanism. The Bilirubin metabolism's rate-

limiting step is this one. A multi-specific organic anion transporter (MOAT), found in the plasma membrane of the bile canaliculi, is the protein in question. MOAT Literature is an orgamcamon transporter that belongs to the ATP binding cassette family. The same medications that stimulate bilirubin conjugation can also induce the hepatic transit of conjugated bilirubin into bile [15]. Thus, conjugation and excretion of bilirubin form a cohesive, functional unit. Glucuronides make up 95% of the glycosidic conjugates excreted in bile from adults, with the remaining glucosides and xylosides making up the remaining 2%. Diglucuronide makes up 90% of the glucuronides, whereas monoglucuronide makes up the remaining 10%. (10 percent).

The gut does not significantly reabsorb bilirubin glucuronides. Instead, they are digested by the -glucuronidase enzyme, which is produced by bacteria, intestinal epithelial cells, and the liver. The anaerobic gut microbial flora subsequently reduces this unconjugated bilirubin to create a trio of colourless tetrapyrroles known as urobilinogen [16]. All bridge carbons in these three bilirubin reduction products are in their saturated (methylene) state. The two end pyrrole rings and the degree of hydrogenation of the vinyl side chains distinguish the urobilinogens from one another. Urobilinogens are stercobilinogen, mesobilinogen, or urobilinogen, depending on how much more hydrogen they contain than bilirubin [17]. The daily production of urobilinogen can reach a maximum of 20%, 20% of which is reabsorbed from the gut and enters the enterohepatic circulation. A tiny portion (2 to 5 percent) of the reabsorbed urobilinogen enters the systemic circulation and manifests as urine after being absorbed by the liver and re-excreted in the bile [18]. The three urobilinogens are spontaneously oxidized in the lower digestive tract at the middle methylene bridge to provide the corresponding bile pigments, stercobilin, mesobilin, and urobilin, orange-brown and the main stools colours. About half of the conjugated bilirubin excreted in bile was converted to substances other than urobilinogens [19]. Jaundice, also known as icterus, is a disorder marked by hyperbilirubinemia and bile pigment deposition in the skin, mucous membranes, and sclera, giving the patient a yellow look. Jaundice-causing deficiencies in bilirubin metabolism can happen at any point along the metabolic route. Typically, the illnesses are categorized as having both conjugated and unconjugated hyperbilirubinemia [20].

7. HISTORICAL PERSPECTIVE

Galen and Hippocrates both recognized jaundice as a sickness indicator, but Bartholomeus Metlinger's paediatric treatise Ein Regiment der Jungen Kinder has the earliest mention of jaundice about neonates. Jaundice has long been understood to be a disease symptom and connected to hepatic function [21]. In 1847, Virchow noticed the buildup of minute yellow crystals in bruises, wound fluid, and subcutaneous hematomas after red blood cells were phagocytosed. This observation provided the first experimental evidence for a link between bilirubin and heme. The most typical ailment among neonates that need medical treatment is jaundice. The buildup of unconjugated bilirubin causes the skin's and sclera's yellowish colouring in neonates with jaundice. Unconjugated hyperbilirubinemia in most babies indicates a typical developmental condition. Unconjugated bilirubin is neurotoxic and can kill neonates and leave children with lasting neurologic sequelae. Therefore it can be dangerous when blood bilirubin levels rise significantly in some infants. Whether it results from physiological or pathological reasons, neonatal hyperbilirubinemia requires adequate and prompt treatment [22].

The foetus can maintain low bilirubin levels while in gestation because the placenta transfers unconjugated bilirubin straight to the mother, with just a minimal contribution from the foetal liver to bilirubin excretion. At around 20 weeks of pregnancy, bilirubin UDPGT is detected, although its levels remain modest until the baby is born. Unconjugated bilirubin is present in trace levels in the bile and intestinal contents of foetuses before 20 weeks of gestation [23]. Severe foetal hyperbilirubinemia later in gestation can trigger the synthesis of an enzyme, improving the fetus's capacity to conjugate bilirubin. It has been discovered that the concentration of bilirubin in the venous blood leaving the placenta is lower than the concentration in the umbilical arteries supplying blood to the placental circulation. By using this method, only unconjugated bilirubin is eliminated. The foetus still has the conjugated bilirubin that was generated [24]. The foetal liver serves as the alternative pathway for bilirubin elimination. Most of the conjugated bilirubin excreted in the foetal intestine is digested and reabsorbed in the foetal circulation. Around 12 weeks into the pregnancy, bilirubin can be discovered in the normal amniotic fluid. Usually, it goes away around 36–37 weeks of pregnancy. The tracheobronchial secretions, foetal membranes, meconium, diffusion through the umbilical veins, diffusion from the skin, and direct transfer from the maternal circulation are all routes by which bilirubin enters the amniotic fluid [25].

8. CONCLUSION

Bilurubin controls intracellular heme levels and functions as a protective antioxidant. A heat shock protein called heme oxygenase 1 is activated as a general response to oxidative stress, which includes

hyperthermia. When guanylate cyclase is activated by the carbon monoxide created during bilirubin production, it can diffuse into nearby cells and generate the intracellular messenger cyclic guanosme monophosphate.

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REFERENCES

[1] Silver GA. Virchow, the heroic model in medicine: health policy by accolade. American journal of public health. 1987;77(1):82-8.
[2] Fevery J. Bilirubin in clinical practice: a review. Liver International. 2008;28(5):592-605.
[3] Adin CA. Bilirubin as a therapeutic molecule: challenges and opportunities. Antioxidants. 2021;10(10):1536.
[4] Vítek L, Tiribelli C. Bilirubin: The yellow hormone?. Journal of Hepatology. 2021;75(6):1485-90.
[5] El-Zohry AM, Diez-Cabanes V, Pastore M, Ahmed T, Zietz B. Highly Emissive Biological Bilirubin Molecules: Shedding New Light on the Phototherapy Scheme. The Journal of Physical Chemistry B. 2021;125(32):9213-22.
[6] Ni SD, Chen YL, Chen YQ, Zhou K, Ding HM. Molecular Simulation Studies on the Interactions of Bilirubin at Different States with a Lipid Bilayer. Langmuir. 2021;37(40):11707-15.
[7] Ziberna L, Martelanc M, Franko M, Passamonti S. Bilirubin is an endogenous antioxidant in human vascular endothelial cells. Scientific reports. 2016;6(1):1-6.
[8] Liu YY, Pan HF, Xu JH, Chen JQ. Long chain fatty acid affects excited state branching in bilirubin-human serum protein Complex. Chinese Journal of Chemical Physics. 2021;34(5):621-7.
[9] Cao X, Zhang C, Gao Z, Liu Y, Zhao Y, Yang Y, Chen J, Jimenez R, Xu J. Ultrafast internal conversion dynamics of bilirubin bound to UnaG and its N57A mutant. Physical Chemistry Chemical Physics. 2019;21(5):2365-71.
[10] Nocentini A, Bonardi A, Pratesi S, Gratteri P, Dani C, Supuran CT. Pharmaceutical strategies for preventing toxicity and promoting antioxidant and anti-inflammatory actions of bilirubin. Journal of Enzyme Inhibition and Medicinal Chemistry. 2022;37(1):487-501.
[11] Cazzola M. Ineffective erythropoiesis and its treatment. Blood, The Journal of the American Society of Hematology. 2022;139(16):2460-70.
[12] Narwal V, Batra B, Kalra V, Jalandra R, Ahlawat J, Hooda R, Sharma M, Rana JS. Bilirubin detection by different methods with special emphasis on biosensing: A review. Sensing and Bio-Sensing Research. 2021;33:100436.
[13] Ruiz AR, Crespo J, Martínez RM, Iruzubieta P, Mercadal GC, Garcés ML, Lavin B, Ruiz MM. Measurement and clinical usefulness of bilirubin in liver disease. Advances in Laboratory Medicine/Avances en Medicina de Laboratorio. 2021;2(3):352-61.
[14] Thomas M, Hardikar W, Greaves RF, Tingay DG, Loh TP, Ignjatovic V, Newall F, Rajapaksa AE. Mechanism of bilirubin elimination in urine: insights and prospects for neonatal jaundice. Clinical Chemistry and Laboratory Medicine (CCLM). 2021;59(6):1025-33.
[15] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Cmaj. 2005;172(3):367-79.
[16] Tiribelli C, Ostrow JD. Intestinal flora and bilirubin. Journal of hepatology. 2005;42(2):170-2.
[17] Ostrow JD, Pascolo L, Brites D, Tiribelli C. Molecular basis of bilirubin-induced neurotoxicity. Trends in molecular medicine. 2004;10(2):65-70.
[18] Roy-Chowdhury N, Wang X, Roy-Chowdhury J. Bile pigment metabolism and its disorders. InEmery and Rimoin's Principles and Practice of Medical Genetics and Genomics 2020;1:507-553.
[19] Bloomer JR, Berk PD, Howe RB, Waggoner JG, Berlin NI. Comparison of fecal urobilinogen excretion with bilirubin production in normal volunteers and patients with increased bilirubin production. Clinica Chimica Acta. 1970;29(3):463-71.
[20] Johnson L, Bhutani VK. The clinical syndrome of bilirubin-induced neurologic dysfunction. Seminars in perinatology 2011;35(3):101-113.
[21] Amodio P. Hepatic encephalopathy: historical remarks. Journal of Clinical and Experimental Hepatology. 2015;5:S4-6.
[22] Gartner LM. Neonatal jaundice. Pediatrics in review. 1994;15(11):422-32.
[23] Schenker S, Dawber NH, Schmid R. Bilirubin metabolism in the fetus. The Journal of Clinical Investigation. 1964;43(1):32-9.
[24] Brown AK. Perinatal aspects of bilirubin metabolism. Obstetrics and Gynecology Annual. 1975;4:191-212.
[25] Brodersen R, Jacobsen J, Hertz H, Rebbe H, Sørensen B. Bilirubin conjugation in the human fetus. Scandinavian Journal of Clinical and Laboratory Investigation. 1967;20(1):41-8.